Study Outline for the Toxicology Branch of the Columbia Environmental Research Center (CERC), USGS, Columbia MO

Study code:	13-20-08
Title:	An evaluation of acute 24-h toxicity of NaCl to glochidia of fatmucket (*Lampsilis siliquoidea*)
Lead Investigator:	Ning Wang
Lead Technician:	Chris Ivey
Date:	February 28, 2013
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Basis+ project number:	NA00EJR
Basis+ task number:	20
USGS program element:	42100
Reviewed by:	Chris Ingersoll and Ed Hammer

Study background

A few previous studies have indicated that glochidia of mussels are sensitive in acute 24-h exposures to NaCl (Bringolf et al. 2007, Valenti et al. 2007, Cope et al. 2008, Gillis 2011). However, the effect concentrations ranged largely within a species between different tests under similar conditions, or within a species tested at different waters (across different hardness of reconstituted waters or various field-collected waters; Gillis 2011).

Objectives of this study are to (1) confirm acuteeffect of NaCl on glochidia of fatmucket (*Lampsilis siliquoidea*) in ASTM reconstituted moderately hard water (hardness about 100 mg/L as $CaCO_3$) used in the tests conducted by Gillis (2011), and (2) evaluate the hardness influence on NaCl toxicity to fatmucket glochidia in the CERC diluted well water of about 50, 100, 200, and 300 mg/L hardness.

Test organism

Gravid female fatmucket were collected in early December 2012 from the Silver Fork of Perche Creek (Boone County, MO). The sampling site has apparently stable mussel populations with multiple age classes. The adult mussels are held in well water (hardness about 300 mg/L as $CaCO_3$, alkalinity 250 mg/L as $CaCO_3$, pH 8.2) at 10°C. The viability of glochidia isolated from each female mussel will be determined before starting a toxicity test. Glochidia will be gently flushed from the marsupial gills of a female mussel into a 300-ml crystallizing dish using a 1-mm needle and 35-ml syringe filled with culture water (300 mg/L hardness). Three subsamples of about 100 glochidia will be impartially transferred to each of three wells of a 24-well polystyrene tissue-culture plate filled with about 2 ml of well water. Glochidia in each well will be examined with a dissecting microscope, and the number of closed glochidia will be recorded. After adding one drop of saturated NaCl solution (about 12 g NaCl in 50 ml of deionized water; Wang et al. 2007) to each well, open and closed glochidia will be counted within 1 min.

Glochidia that closed in response to NaCl will be classified as alive (or viable), whereas glochidia that are closed before the addition of NaCl or that remained open after the addition of NaCl will be classified as dead (nonviable). Survival (viability) of glochidia will be calculated as described in ASTM (2012a):

Survival (%) = 100 x (number of closed glochidia after adding NaCl solution - number of closed glochidia before adding NaCl solution) ÷ (total number of open and closed glochidia after adding NaCl solution).

If the survival of glochidia from an individual mussel is >80% (preferably >90%; ASTM 2012a), the remaining glochidia from that mussel will be used for toxicity testing. Glochidia isolated from three to six mussels will be pooled and mixed to obtain organisms to start a toxicity test. Glochidia will be acclimated to a mixture of 50% culture water (well water) and 50% test water (details follow) that is gradually adjusted to the test temperature over 2 h before the start of a toxicity test.

Toxicity test

Test conditions and procedures will be in accordance with standard methods (ASTM 2012a). The ASTM moderately hard water will be prepared by adding reagent-grade salts ($CaSO_4 \cdot 2H_2O$, $MgSO_4$, KCl, and $NaHCO_3$; EM Science, Gibbstown, NJ, USA) into deionized water following ASTM guidance (ASTM 2012b). The four diluted well waters containing different hardness will be prepared by diluting well water (about 300 mg/L as $CaCO_3$) with deionized water to a hardness of about 50, 100, and 200 mg/L as $CaCO_3$, respectively. Laboratory-grade NaCl (99.9% purity, Sigma-Aldrich, St. Louis, MO) will be used to prepared NaCl concentrations. Test solutions will be prepared as described by Gillis (2011): water will be spiked with NaCl (nominal concentrations of 0, 0.16, 0.32, 0.63, 1.25, 2.5, 5, and 10 g NaCl/L) and held in the dark at 4 °C for 48 h before initiation of an exposure.

Each test will consist of three replicates and will be conducted under static conditions in 300-ml glass beaker containing about 100 ml of test solution. At the beginning of each test, about 500 glochidia will be transferred from the pooled sample of glochidia into each replicate chamber. Test chambers will be held in temperature-controlled water baths at 20±1 ºC. Ambient laboratory light of about 500 lux will be used with 16:8 h light:dark photoperiod.

Initial survival of glochidia for each toxicity test will be estimated by determining the viability of glochidia in three replicate controls at the beginning of the test. A mean viability value will be used to adjust the survival of glochidia after 24 h of exposure (i.e., percent of the initial mean control survival). At each exposure time interval, a subsample of about 100 individuals with 2 ml of test water will be taken from each replicate chamber and transferred into one well of a clean 24-well tissue-culture plate. One drop of the saturated NaCl solution will be added into the well, and the response of glochidia (valve closure) within 1 min will be recorded as described previously. A saturated KCl solution will also be used to determine glochidia viability in at least one test (e.g., the test in ASTM water) to evaluate any potential influence of the use of NaCl or KCl solution on viability determination. The acceptability criterion for a glochidia test is ≥90% control survival at the end of the 24-h exposures.

Water quality (dissolved oxygen, pH, hardness, and alkalinity) will be determined in the control, medium, and high concentrations at the beginning and the end of tests. Salinity and conductivity will be also measured in each exposure concentration at the beginning and the end of tests. Water samples major cations (calcium, potassium, magnesium, and sodium) and major anions (chloride and sulfate) will be collected the control waters at the start of exposures. The cation samples will be stabilized within 24 hours by adding concentrated nitric acid (16 M) to each sample at a volume proportion of 1:100 (1% v/v). Water samples for chloride measurement will be collected from each exposure concentration at the beginning of the test.

Data analysis

Median effect concentrations (EC50) will be determined using Toxicity Relationship Analysis Program (TRAP; Erickson 2010).

References

American Society for Testing and Materials (ASTM). 2012a. ASTM International standard guide for conducting early life stage toxicity tests with fishes (E12421-05). Annual Book of ASTM Standards Volume 11.06, West Conshohocken, PA.

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Table 1. Summary of test conditions for conducting toxicity tests with glochidia in basic accordance with ASTM (2012a)

Test species:	Fatmucket (*Lampsilis siliquoidea*)
Glochidia collection:	Flush mussel gills with water from syringe
Test chemical:	NaCl
Test type:	Static
Test duration:	24 h
Temperature:	20±1°C
Light quality:	Ambient laboratory light
Light intensity:	About 500 lux
Photoperiod:	16:8 h light:dark
Test chamber:	300 ml glass beaker
Test solution volume:	100 ml
Renewal of solution:	None
Age of test organism:	<2 h after releasing from marsupium
Organisms/chamber:	About 500
Replicates/concentration:	3
Feeding:	None
Aeration:	None
Dilution water:	(1) Reconstituted ASTM moderately hard water (hardness 100 mg/L as $CaCO_3$; ASTM 2012b) (2) CERC well water (hardness about 300 mg/L), and three diluted well water (50, 100, and 200 mg/L hardness)
Dilution factor:	0.5
Test concentration:	0, 0.16, 0.32, 0.63, 1.25, 2.5, 5, and 10 g NaCl/L
Chemical residues:	Water samples for chloride analysis from each concentration at the beginning of the test (5 waters x 7 chloride concentration/water). Major ions in each of the five control waters.
Water quality:	Measure dissolved oxygen, pH, hardness, and alkalinity at the control, medium, and high exposure concentrations, and salinity and conductivity at all concentrations at 0 and 24 h
Endpoint:	Survival (valve closure with the addition of NaCl)
Test acceptability:	≥90% survival in controls

Appendix 1. Waterbath request form.

1. Investigator/technician:	Ning Wang/Chris Ivey
2. Study code:	13-20-08
3. Date of request:	February 19, 2013
4. Chemical(s) or Treatment(s):	NaCl
5. Test organism(s):	Fatmucket (*Lampsilis siliquoidea*)
6. Test organism culture:	Adult mussels are held in flow-through tank containing well water at 10°C and fed algal mixture once every other day
7. Temperature (°C):	20
8. Proposed Wet Lab:	RAS
9. Number of small diluters:	1
10. Number of large diluters:	0
11. Number of Hamilton pumps:	0
12. Number, type, size of chambers:	200-ml glass dishes (4 replicates x 7 treatments x 5 waters =140)
13. Number and type water splitter:	0
14. Water type:	ASTM 100 hard; Diluted well water (50, 100, 200, and 300 mg/L hardness as $CaCO_3$)
15. Daily volume water use:	About 200 L
16. Effluent discharge:	Red Line (City)
17. Start date (including set up):	March 2013
18. Exposure duration:	24 hours
19. End date (after cleaning and rehab to original condition):	Late March 2013
20. Flexibility in start date:	None
21. Special needs:	None
22. Response from Ingersoll:	Diluter #1 in the RAS wet lab was assigned for this project (CGI February 28, 2013)